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Regular paper

Hydrogen peroxide is formed upon cooking of vegetables

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Hydrogen peroxide (H_2O_2) is generated under autoxidation of some components of beverages including flavonoids and ascorbate, especially in tea and coffee. As polyphenols are also present in solid food, especially in vegetables, we checked whether hydrogen peroxide is generated during cooking of several common vegetables. The formation of hydrogen peroxide was found in the decoctions of all cooked vegetables studied except for potato and in the homogenates of cooked vegetables except for garlic and purple potato. The highest concentration of hydrogen peroxide in 1:2 (w/v) homogenates was found for the broad bean (73.4±9.0 μM) followed by broccoli (18.6±0.3 μM), onion (10.4±1.6 μM) and leek (10.0±0.3 μM), while the H_2O_2 concentration in the decoctions was the highest for broccoli (24.4±0.8 μM), then for broad bean (21.4±1.1 μM), carrot (13.2±0.2 μM) and cauliflower (12.6±1.2 μM).

Keywords: hydrogen peroxide, vegetables, broad bean, broccoli, onion

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Abbreviations: H_2O_2, Hydrogen peroxide

INTRODUCTION

Endogenous hydrogen peroxide is a reactive oxygen species, generated by activated phagocytes to kill ingested microorganisms (Clifford & Repine, 1982; Thomas, 2017) and by other cells for local signaling. Its elevated concentrations cause apoptosis and cell death (Sies, 2017; Lin *et al*., 2019). Hydrogen peroxide solution (3%) is used externally as disinfectant (Neely & Maley, 1999); virucidal efficiency of H_2O_2 vapor has also been previously demonstrated (Goyal *et al*., 2014). Concentrated H_2O_2 is used as a bleach, i.e., for hair discoloration (Watt *et al*., 2004; Smith *et al*., 2017). H_2O_2 is used for tooth whitening (Alkahtani, *et al.* 2020) and as a component of some mouthwashes (Ortega *et al*., 2020; Vergara-Buenaventura & Castro-Ruiz, 2020). Cases of poisoning with H_2O_2 have been reported (Watt *et al*., 2004) but low doses of H_2O_2, when ingested as solutions of low concentration, may not be toxic at all or may even have a beneficial action, mainly due to the antimicrobial activity. Interestingly, H_2O_2 ingestion has been used for treatment of dementia and enhancement of the immune system function and has been claimed to have anticancer effects and to promote well-being (Yutsis, 2003). Oral exposures to 3% H_2O_2 were reported to be mostly asymptomatic, with only one child among 670 patients developing multiple gastric ulcers and duodenal erosions (Henry *et al*., 1996). However, there are concern that H_2O_2 contained in the mouthwash may be cancerogenic (Consolaro, 2013).

In the context of controversy concerning the health effects of low doses of ingested hydrogen peroxide, it is of interest to have a full picture of natural ingestion of H_2O_2 in food and beverages. Humans ingest H_2O_2 already as newborns since fresh human milk contains about 30 μM H_2O_2; in addition, baby saliva contains ca 10 μM H_2O_2 so the milk entering the digestive tract contains about 40 μM H_2O_2, sufficient to inhibit growth of the opportunistic pathogens like *Staphylococcus aureus* and *Salmonella* spp. (Al-Shehri *et al*., 2015). Later on, we ingest H_2O_2 in commonly consumed beverages, such as tea, coffee and cocoa. These beverages contain flavonoids and phenolic acids (Bubols *et al*., 2013; Galleano *et al*., 2010) which have many beneficial health effects, possibly related to their antioxidant activities (Chu *et al*., 2017; Ullah *et al*., 2020).

However, flavonoids and phenolic acids autoxidize in aerated solutions reacting with oxygen. H_2O_2 is generated as one of products of autoxidation of these compounds; therefore, tea and coffee (Akagawa *et al*., 2003; Grzesik *et al*., 2019, Long *et al*., 1999) and to a lesser extent cocoa and wine (Chai *et al*., Long *et al*., 1999; Tama *et al*., 2022) contain some amounts of H_2O_2, which usually increase with time after their preparation. H_2O_2 is also generated in formulated beverages due to oxidation of ascorbic acid and some other redox-active components (Bopitiya *et al*., 2021).

Flavonoids and other autoxidizing compounds are present not only in beverages, but also in solid food of plant origin, especially in vegetables, and can be expected to generate H_2O_2 in these products as well. The aim of the present study was to examine whether H_2O_2 is generated in cooked vegetables.

MATERIALS AND METHODS

Xylenol Orange was obtained from POCh (Gliwice, Poland). Other reagents were purchased from Merck (Poznań, Poland). Absorptiometric measurements were done in a Stark multimode microplate reader (Tecan Group Ltd., Männedorf, Switzerland), using Greiner 96-well flat-bottom clear polystyrene plates.

Vegetables (broad bean *Vicia faba* L., onion *Allium cepa* L., garlic *Allium sativum* L., leek *Allium ampeloprasum* L., cabbage *Brassica oleracea* L., broccoli *Brassica oleracea var. italica* Plenck, cauliflower *Brassica oleracea var. botrytis* L., carrot *Daucus carota subsp. sa-tivus* (Hoffm.) Schübl. & G. Martens, carrot *Daucus carota ssp. sativus* and potato *Solanum tuberosum* L.) were purchased in a

local supermarket in Rzeszów. Ten grams of a vegetable were added with 20 mL of 50 mM phosphate buffer, pH 7.4, deionized water or tap water and heated in an open falcon tube at 95°C for 10 minutes. Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). A sample of decoction was withdrawn, and the remaining fraction was homogenized with the cooked vegetables in a blender. A sample of the homogenate was withdrawn, centrifuged, and the supernatant was collected. The remaining part was incubated at room temperature (21±1°C) and samples were collected and centrifuged after 3 h and 24 h.

The H_2O_2 concentrations in the decoctions and supernatants of fresh and incubated homogenates was essayed with Xylenol Orange (Gay & Gebicki, 2003; Grzesik *et al.*, 2019) in duplicate aliquots, one of them being incubated with catalase (10 μg/mL) for 15 min prior to the assay. The difference between the results obtained for both aliquots was assumed to represent the H_2O_2 concentration in the sample. This method may underestimate the H_2O_2 concentration but ensures full specificity for this compound.

The experiments were run in triplicate and were repeated at least three times. Statistical significance of differences was estimated using one-tailed Student's *t*-test.

RESULTS

The presence of H_2O_2 was detected in the decoctions of all studied cooked vegetables with the exception of potato and in the homogenates of all studied cooked vegetables except for garlic. The highest concentration of H_2O_2 in 1:2 (w/v) homogenates was found for the broad bean (73.4±9.0 μM) followed by broccoli (18.6±0.3 μM), onion (10.4±1.6 μM) and leek (10.0±0.3 μM). In the decoctions, the highest H_2O_2 concentration was recorded for broccoli (24.4±0.8 μM) and then for broad bean (21.4±1.1 μM), carrot (13.2±0.2 μM) and cauliflower (12.6±1.2 μM).

Incubation of the homogenates of cooked vegetables for 3 and 24 h generally decreased the concentration of H_2O_2, sometimes (carrot, garlic, cabbage) to zero values (Fig. 1).

When the vegetables were cooked in deionized or tap water, the formation of H_2O_2 was lower than upon cooking the phosphate buffer, pH 7.4, but still occurred,

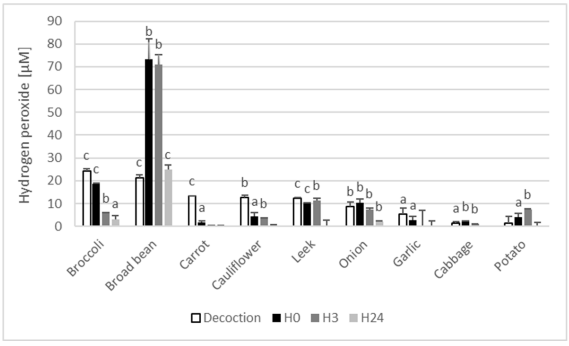

Figure 1. Generation of hydrogen peroxide in cooked vegetables.
H0 – homogenate, immediately after cooking, H3 – homogenate after 3 h incubation, H24 – homogenate after 24 h incubation; [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ with respect to zero (Student's *t*-test).

as reported for the broad bean (Table 1). This decreased generation of H_2O_2 in water is due to the lower pH of the homogenate and decoction of vegetables (Table 2).

DISCUSSION

The presence of H_2O_2 in cooked vegetables has not been reported to our best knowledge. There is no reason to suspect the presence of significant amounts of H_2O_2 in fresh vegetables since the catalase and peroxidase activities remove H_2O_2 inevitably produced in the living cells. However, when these activities are inactivated by cooking and redox-active compounds become exposed to ambient oxygen, this equilibrium is disrupted and H_2O_2, a by-product of autoxidation of polyphenols, can accumulate. The formation of H_2O_2 occurred at the highest rate during cooking at high temperature; upon cooling and subsequent incubation, the H_2O_2 concentrations in the vegetable homogenates decreased rather than increased, apparently due to reactions with components of the homogenates such as released heme and flavonoids themselves, which apart from generating H_2O_2, are also able to react with this compound (Bi *et al.*, 2014; He *et al.*, 2018).

Especially high H_2O_2 generation was found in the fava beans (Fig. 1), known to contain considerable amounts of pyridine derivatives, vicine and convicine, constitut-

Table 1. Generation of hydrogen peroxide in the decoction and homogenate of broad bean cooked in phosphate buffer, pH 7.4, tap water and deionized water.

Hydrogen peroxide [μM]			
Tested material	Phosphate buffer	Tap water	Deionized water
Decoction	53.5±2.3[c]	15.4±0.6[c]	19.3±1.5[b]
Homogenate 0 time	76.0±3.8[c]	28.1±4.8[b]	22.8±2.2[a]
Homogenate, 3 h	61.7±3.5[b]	29.5±7.0[a]	8.0±4.3[a]
Homogenate, 24 h	9.0±1.1[b]	10.0±1.6[b]	8.5±1.8[b]

[a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$ with respect to zero (Student's *t*-test)

Table 2. pH of decoction and homogenate of broad been cooked in the phosphate buffer, tap water and deionized water.

Tested material	Prepared in buffer	Prepared in tap water	Prepared in deionized water
Decoction	7.05±0.02	6.85±0.03	6.41±0.04
Homogenate 0 time	7.15±0.03	6.87± 0.04	6.60±0.02

pH was measured after cooling to the room temperature

ing up to 0.5% of the total weight of the bean. Their aglycones autoxidize readily and, upon ingestion, undergo redox cycles, producing large amounts of H_2O_2 and hemolysis in sensitive individuals (favism) (Halliwell & Gutteridge, 1999). The present results demonstrate that significant generation of H_2O_2 by fava beans takes place also during cooking, under the conditions of limited redox cycling. Moreover, fava beans contain many flavonoids, especially catechin and rutin, and phenolic acids (Johnson *et al.*, 2021); all these compounds can generate H_2O_2 upon autoxidation (Grzesik *et al.*, 2019).

It should be pointed out that the concentrations of H_2O_2 formed in vegetables may be underestimated by our method since some flavonoids inhibit catalase (Krych & Gebicka, 2013) used to warrant specificity of the assay for H_2O_2.

Oxidation of flavonoids and many other compounds is pH-dependent and decreases as the pH is lowered (Akagawa *et al.*, 2002; Arakawa *et al.*, 2004; Grzesik *et al.*, 2019). Elevated pH facilitates oxidation of phenolic compounds including flavonoids since the phenol groups dissociate at higher pH. The electron on the deprotonated phenol group reduces oxygen forming superoxide radical anion $O_2^{-\bullet}$ and a phenoxyl radical. Superoxide can be further reduced by the phenoxyl radicals (oxidizing a phenoxyl radical to a quinone) or dismutate; both reactions generate H_2O_2 (Arakawa *et al.*, 2004; Grzesik *et al.*, 2019). The presence of transition metals, especially iron, ions facilitate autoxidation of phenolic compounds (Grzesik *et al.*, 2019), which explains higher H_2O_2 concentrations in the homogenates of fava beans cooked in tap water rather than in deionized water (Table 1).

We compared H_2O_2 generation when cooking various vegetables in phosphate buffer, pH 7.4, to provide identical conditions for comparison. Cooking vegetables in deionized or tap water, resulting in decoctions and homogenates of lower pH (Table 2), decreased the rate of H_2O_2 generation but still resulted in the production of significant amounts of H_2O_2 (Table 1). Thus, an obvious means to reduce its generation during cooking is to keep low pH and avoid transition metal ion contamination during cooking.

The generation of H_2O_2 at low concentrations in some cooked vegetables does not appear to raise health problems and may even have beneficial effects. H_2O_2 can exert bactericidal and virucidal action, and thus contribute to the mouth hygiene and health. High H_2O_2 concentrations may damage colon cells (Wijeratne *et al.*, 2005; Pravda, 2020), but low concentrations were reported to have physiologic effects on intestinal water and electrolyte transport (Karayalcin *et al.*, 1990) and suggested to stimulate cell divisions in the damaged intestine, thus contributing to epithelial repair (Craven *et al.* 1986). In the digestive tract, H_2O_2 can react with available iron, form the hydroxyl radical and other free radicals, and facilitate digestion as proteins subjected to free radical action may show enhanced susceptibility to proteolytic enzymes (Wolff & Dean, 1986).

CONCLUSION

Cooking vegetables generates hydrogen peroxide both in the decoction and inside the solid food. These small amounts of hydrogen peroxide formed do not seem to pose a health danger and may even exert beneficial effects.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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